The assembly of *C. elegans* lamins into macroscopic fibers

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Abstract

Intermediate filament (IF) proteins are known mainly by their propensity to form viscoelastic filamentous networks within cells. In addition, IF-proteins are essential parts of various biological materials, such as horn and hagfish slime threads, which

exhibit a range of mechanical properties from hard to elastic. These properties and their self-assembly nature made IF-proteins attractive building blocks for biomimetic and biological materials in diverse applications. Here we show that a type V IF-protein, the *C. elegans* nuclear lamin (Ce-lamin), is a promising building block for protein-based fibers. Electron cryo-tomography of vitrified sections enabled us to depict the higher ordered assembly of the Ce-lamin into macroscopic fibers through the creation of paracrystalline fibers, which are prominent in vitro structures of lamins. The lamin fibers respond to tensile force as other IF-protein-based fibers, i.e., hagfish slime threads, and possess unique mechanical properties that may potentially be used in certain applications. The self-assembly nature of lamin proteins into a filamentous structure, which is further assembled into a complex network, can be easily modulated. This knowledge may lead to a better understanding of the relationship in IF-proteins-based fibers and materials, between their hierarchical structures and their mechanical properties.

1. Introduction

Biological materials execute diverse mechanical tasks in nature (Chen et al., 2008). Through evolution, their building blocks and structural design have been optimized in order to cope with a variety of environmental factors and tasks. The study of biological materials and biomimetic materials has gained interest, particularly for their potential use as alternatives for a variety of materials required by humans, especially in regenerative medicine and medical devices (Miserez et al., 2015). Among biological materials, protein-based fibers are under intense scrutiny for their

exceptional mechanical properties, which combine elasticity and toughness (Gosline et al., 2002; Miserez and Guerette, 2013). Biological fibers such as spider and cocoon silks are widely used as biological materials in diverse applications (Omenetto and Kaplan, 2010). They have been elucidated in great detail in terms of structure and mechanical properties (Lintz and Scheibel, 2013). However, a complete description of their hierarchical structure and assembly pathway is still underway (van Beek et al., 2002). Another example is the native hagfish slime threads (Winegard et al., 2014), which have been shown to possess mechanical properties that are comparable to spider silk fibers (Negishi et al., 2012). The micrometer-sized slime threads are built from two intermediate filament (IF) proteins (α and γ) that are homologous to epidermal keratin (Koch et al., 1995). Due to the combination of impressive mechanical performance and the self-assembly nature of IF-proteins (Herrmann and Aebi, 2004), the slime threads have been proposed as biomimetic models for creating protein fibers and biomaterials with remarkable mechanics (Fudge et al., 2010). Thus, a study on vimentin, a type III cytoplasmic IF protein, showed that it can be assembled into tough fibers (Pinto et al., 2014) that, with further optimization, can reach the extraordinary mechanical properties of native hagfish threads. An improvement in fiber strength was achieved by self-assembly of recombinant vimentin into native-like 10-nm filaments, a prominent structure formed by cytoplasmic IF-proteins. It was suggested that a secondary structure transition of α -helix to β -sheets is a major contributor to strength in IF native fibers, similar to slime threads and keratin fibers (Fudge et al., 2003; Kreplak et al., 2004). In this study, we used the *C. elegans* lamin (Ce-lamin) as a potential building block for new macroscopic protein-based fibers (Karabinos et al., 2003).

Lamins are classified as being either A or B type (Dittmer and Misteli, 2011). Together they form a composite filaments network at the periphery of the nucleus, the nuclear lamina (Aebi et al., 1986; Shimi et al., 2008), which functions as an elastic shell (Dahl et al., 2004; Rowat et al., 2005). In vitro studies show that lamins, like all IF-proteins, consist of a central α -helical coiled-coil domain flanked by a non- α -helix 'head' and 'tail' domain. Two central α -helical domains interact to form a lamin dimer (Stuurman et al., 1998). Furthermore, lamin dimers from diverse organisms tend to form paracrystalline fibers in vitro (Heitlinger et al., 1991; Moir et al., 1991; Sasse et al., 1998). Interestingly, Ce-lamin, similar to that of mammalian lamins, forms paracrystalline fibers. In general, paracrystalline arrays have been observed in other assemblies of fibrous proteins such as collagen (Kadler et al., 1996). The variety of banding patterns that have been observed in these assemblies is mainly dependent on protein sequence, structural domains, and assembly pathways. Paracrystalline fibers assembled from lamins have been studied intensively using electron cryotomography (Bank et al., 2011; Ben-Harush et al., 2009; Taimen et al., 2009). These studies suggest that paracrystalline fibers are assembled in four hierarchical assembly steps (Fig. 5). First, lamin monomers coiled around each other to form a coiled-coil dimer, which is ~55 nm in length and ~2 nm thick. These dimers associate in head-to-tail fashion, with a short overlap, to form a head-to-tail polymer of dimers. Along the head-to-tail polymer axis, the distance between dimeric tails is around 48 nm (Ben-Harush et al., 2009; Stuurman et al., 1998). Next, two head-to-tail polymers interact laterally and in antiparallel fashion to form tetrameric protofilaments, ~4 nm diameter in cross-section. The distance between dimeric tails along the protofilament alternates between 21 to 27 nm, which is the result of staggered association between two head-to-tail polymers of dimers. The last step is the lateral association of protofilaments

into Ce-lamin filaments or paracrystalline fibers. Therefore, the banding pattern along the paracrystalline fibers represents the distance between the C-terminus tail domains.

Ce-lamin forms stable 10-nm IF-native-like filaments, in which the proteins arrange similarly as in paracrystalline fibers. Hence, structural elements of paracrystalline fibers assemblies might keep one of the native-like structures of lamin filaments. Here, we used conditions favoring the self-assembly of Ce-lamin into paracrystalline fibers to create a network of microfibers, which then were mechanically spun into tough macroscopic fibers. Paracrystalline lamin fibers were further assembled into macroscopic fibers that resembled vimentin fibers and native hagfish threads in their response to tensile force. Therefore, simple Ce-lamin preparation might serve as a platform to better understand the relationship between hierarchical structure and mechanical properties in IF-based materials.

2. Material and Methods

2.1. Ce-lamin expression and purification

Ce-lamin was expressed using BL21DE3 Rosetta plysS bacteria containing plasmid encoding *lmn-1* (NC_003279.8), and was induced using IPTG. Three hours after induction, bacteria were harvested by centrifugation. The resulting pellet was suspended in a re-suspension buffer (20 mM Tris-HCl pH=7.6, 200mM NaCl, 1mM EDTA, 1% v/v Triton and 1:10,000 v/v Calbiochem Protease Inhibitor Cocktail Set III) . The bacterial suspension was sonicated, inclusion bodies were washed twice with the re-suspension buffer, and then incubated with 20 unit/mL Benzonaze Nuclease (Novagen, Denmark) for 30 min. Inclusion bodies were centrifuged again at 8,000 g for 10 min, then dissolved in a urea buffer (20mM Tris-HCl pH=7.6, 50mM

NaCl, 6M urea). Lastly, the suspension was centrifuged at 16,000 g, for 1 h at 4°C and the supernatant was kept for SDS-PAGE analysis and further use.

2.2. Protein assembly and preparation of lamin fibers

Ce-lamin (1.0 mg/ml) in a 6M urea-containing buffer was dialyzed against 15 mM Tris-HCl, pH 7.6, 1 mM DTT for 2 h at room temperature, followed by a 3 h dialysis against 25mM Tris-HCl, pH 9.0, 20mM CaCl₂, 1 mM DTT. Throughout the dialysis process the orientation of the dialysis bags was kept in the vertical position, which was a crucial matter for the creation of a long microfibers network that facilitates fiber spinning (when the dialysis bag is horizontal, aggregation of paracrystalline fibers is formed at the second dialysis step). Next, the dialysis bags were exposed to isopropanol for 5 minutes, while the macroscopic fibers were spun out of the solution in a rotating manner and kept in the dialysis buffer (non-drawn fibers). For cryo-sectioning, the microfibers were spun down at the end of the second dialysis stage at 4000 g, for 10 min.

2.3. Mechanical characterization of Ce-lamin fibers

Fibers were mounted onto a cardstock paper frame and glued at both ends using super glue (Scotch). Fiber length was measured using a standard ruler. Tensile testing was performed using an Instron single column universal testing machine (Instron model 3345) with a 100 N load cell, at room temperature, 50% humidity, and a crosshead speed of 0.3 mm/min. All wet fibers (n = 8) were dried by pulling during testing until an elevation in stress was observed. Fiber diameters were measured using SEM images of post-testing fibers. Therefore, breaking stress was calculated as the engineering stress at failure, and breaking strain was calculated as the strain (d_l/L_o) at failure. Young's modulus was calculated as the slope of the stress-strain curve at the

elastic region of the curve prior to the yield point. Toughness at failure was calculated by measuring the area under the stress-strain curve.

2.4. FTIR measurements

Nicolet spectrometer with MCT detector (nitrogen cooled detector) was used. The fibers were mounted on zinc selenide crystals that are transparent to infrared radiation. Using the microscope attached to the infrared spectrometer, we carefully choose the measured areas, which were not in the edge sites. The measured spot has a square shape with side length of 100 μm . The measurements were performed in the transmission mode in the spectral range of 650-4000 cm^{-1} . Each spectrum was measured for 45 seconds resulting on 128 co-added scans for each individual spectrum.

In order to compare among the spectra measured from different fibers, sited and days all the spectra were manipulated using OPUS 7 software (BRUKER Germany). All the spectra were smoothed using Savitzky-Golay algorithm 13 points. The smoothed spectra were cut in the amide I region 1600-1720 cm^{-1} ; baseline corrected using "concave rubberband" method with 5 sections and 2 iterations. Using rubberband method the spectrum was divided into 5 regions, a polynomial fit was performed using the minima in all the sections and then subtracted from the original spectrum. This procedure was repeated as the number of iteration (2 times in our case). The baseline corrected spectra were normalized using min-max normalization. Using min-max normalization the minimum y-value becomes zero and the maximum y-value in the specific range is expanded in the y-direction to 2 absorbance units.

2.5. Optical polarization microscopy

Fibers were deposited onto a clean glass microscope slide. Samples were viewed on an Olympus SZX9 microscope at x10 magnification.

2.6. Scanning electron microscope (SEM)

Electron microscope images were acquired using a Zeiss Ultra55 high resolution scanning electron microscope (HRSEM). Samples were mounted on the SEM holder using double-sided carbon tape. In order to avoid charging, low voltage (1kV) and low current were set as the working conditions.

2.7. Cryo-sectioning of Ce-lamin microfibers

Lamin microfibers were spun down to the bottom of a 2.0 ml tube. Samples were gently dislodged from the bottom of the tube with a toothpick and the preparation buffer (second dialysis buffer) was exchanged for the fixative mixture. The mixture included 2% formaldehyde and 2.5% glutaraldehyde; the samples were left in the mixture for 2 hours. The time and composition were adjusted to the thickness of the sample. Both fixatives were prepared in the preparation buffer. Post fixation, the hardened pellets were chopped into small cubes of approximately 1mm³ and transferred into a new tube with 2.5 M sucrose, in water. Samples were infiltrated with sucrose overnight at 4°C with gentle rotation. A single sample cube was subsequently frozen on a cryo-ultramicrotome aluminum pin by immersion into liquid nitrogen. This was repeated several times in order to produce several samples for cryo-sectioning. Trimming was carried out at -75 °C with a 100 nm feed at a speed of 40 mm/s, using a trimming knife (Cryo trim 45°, Diatome). Cryo-sectioning was carried out at a speed of 0.4-0.6 mm/s at -105 °C using a sectioning knife (Cryo immuno 35°, Diatome). Sections of 200 nm were collected from the cutting knife as five-section ribbons, picked up with a droplet of 2.3 M sucrose, applied to plasma-cleaned holey carbon-coated copper grids (Quantifoil), and left rehydrating in a pool of water for 2 hours. Standard gold markers (BSA gold tracers, 10 nm, Aurion, EMS)

were applied to the grids, followed by plunging them into liquid-nitrogen-cooled liquid ethane and transferring them to be stored (Bokstad et al., 2012).

2.8. Electron cryo-tomography

Five cryo-tomograms were acquired using a 300 kV Titan Kryos transmission electron microscope (FEI) equipped with a Gatan post-column energy filter and a K2 summit director electron detector. Tilt series were acquired, covering an angular range of -60° to 60° ; with 2° tilt increments and a defocus of $-6\ \mu\text{m}$, resulting in a pixel size of 0.34 nm at the specimen level. Projection images were aligned to a common origin using fiducial gold markers, and reconstructed by means of weighted back-projection, as implemented by the TOM toolbox software package (Nickell et al., 2005)

3. Results and Discussion

3.1. Ce-lamin fiber formation

Purification of the Ce-lamin protein from bacteria requires isolation under denaturing conditions. Thus, recombinant lamins expressed in *Escherichia coli* are deposited in ‘inclusion bodies’ and are isolated by high molar concentrations of chaotropic substances, e.g., urea. Therefore, the formation of Ce-lamin paracrystalline fibers is promoted by two assembly dialysis steps, in which divalent ions (CaCl_2) are used in the second step to yield paracrystalline fibers. Here, a network of microfibers was detected 15 minutes after lamins were introduced to the divalent ions, and as the dialysis proceeded the network of microfibers was organized into a hollow cylinder-like structure (Fig. 1A and Fig. S1). Namely, the network of microfibers grew longitudinally with a defined thickness. The assembly mechanism by which Ce-lamin assembles into paracrystalline fibers that grow along the long axis of the dialysis bag

and not inward is not clear. It might that the dialysis bag supports, at first, the buildup of the network.

In order to verify that a network of lamin microfibers was assembled from paracrystalline fibers, electron cryo-tomography of a vitrified section was applied to such preparations (Fig. 1A), demonstrating that, indeed, a lamin microfibers network is built from non-uniform paracrystalline assemblies that are interconnected at random orientations. This was also demonstrated by birefringence analysis of the dry non-drawn fibers (Fig. 2B). The lengths of the paracrystalline fibers vary from 350 to 1500 nm and their diameters vary from 30 to 200 nm. At the assembly conditions used here, it might that shorter and longer fibers exist, but it seems that the majority of the lengths and diameters of paracrystalline fibers lie at that range. The reason for the size limitation is not clear, although it is probably due the kinetics of the dimers assembly at the assembly condition that was used in this study. Other assembly conditions may lead to different organization and sizes of paracrystalline fibers within the microfibers network. Moreover, the banding pattern along the paracrystalline fibers alternates, on average, between 21 to 27 nm, which is identical to the banding pattern observed in individual paracrystalline fibers (Ben-Harush et al., 2009).

Next, we pull the network using a rotational movement to form the macroscopic Ce-lamin fiber (Fig. 1B), indicating that lamin wet macroscopic fibers are assembled from paracrystalline fibers. Hence, we conclude that the Ce-lamin macroscopic fibers are built from coiled-coil dimers that are assembled into higher-order structures that self-organized into paracrystalline fibers and eventually into a network of microfibers, suggesting that Ce-lamin wet fibers keep the structural integrity of lamins filaments,

which probably retain the special mechanoelastic properties of intermediate filament proteins.

3.2. The structural organization of Ce-lamin macroscopic fibers

To learn about the mechanical properties of the macrofibers, Ce-lamin macroscopic fibers went through three pull procedures, with the first pull designed to dry the fiber (non-drawn fibers) under a relatively constant tension force in order to avoid contraction (pre-drawn fibers). Fig. 2A shows that the fiber surface was relatively rough after the first pull and that it is built from straight microfibers. These results could indicate that the low force (Fig. 4) applied to the non-drawn fibers during the drying process only aligns the microfibers along the long axis of the macroscopic fiber. In addition, birefringence analysis demonstrates the lack of orientation within pre-drawn fibers. This might mean that while the microfibers are relatively aligned along the long axis of the fiber, the paracrystalline fibers within it are still randomly oriented.

The second pull was applied to the pre-drawn fibers until their breakage (single drawn fibers). The morphology of the single drawn fibers was smoother than the pre-drawn fibers and their birefringence was larger than that of the pre-drawn fibers, which could stem from additional compaction of the microfibers, and the reorientation and slippage of paracrystalline fibers within the microfibers. Other possible structural rearrangement may be focused on the slippage and compaction of protofilaments within paracrystalline fibers. Possible sliding of protofilaments may lead to intermolecular cross-link breakage. The divalent ion is known to prompt the assembly of paracrystalline fibers, probably by the lateral association of protofilaments.

Therefore, sliding of protofilaments within paracrystalline fibers may lead to irreversible deformation of the paracrystalline fibers, and thus the fiber.

The third pull was applied to the single drawn fibers until breakage (double drawn fibers). The morphology of the double drawn fibers was a nanofibril-like structure (Fig. 2A), and their birefringence was larger than that of the single drawn fibers (Fig. 2B). It might be that additional structural arrangement, such as slippage of head-to tail polymers within protofilaments, which is associated with coiled-coil dimers sliding along the long axis of the protofilaments. Moreover, infrared absorption spectra of non- and double drawn fibers in the amide I region 1600-1720 cm^{-1} (Fig. 3), which is sensitive to secondary structure of proteins, demonstrate that the amide I absorption bands are larger in the double drawn fiber and has a clear shoulder in the 1670-1715 region. This shoulder may be due to high frequency vibrations of an antiparallel β sheets of amide I (Eckel, 2001). This analysis may suggest that uncoiling of the coiled-coil dimer has taken place concurrently with transition of the secondary structure to exhibit a more uniform surface. Such behavior has been previously shown for other IF-protein-based fibers. During the three pulling procedures, the possible compaction and slippage of paracrystalline fibers, protofilaments, and head-to tail polymers of dimers, is the main reason for the shrinkage in the diameters of the fibers (Table 1).

3.3. Stress-strain properties of Ce-lamin fibers

Stress-strain curves of single and double drawn fibers show mechanical behavior similar to dry vimentin fibers and native hagfish slime thread (Fig. 4). This demonstrates a common response of IF-protein assemblies to tensile force, where the

elastic range of the single drawn dry fibers is relatively short (~5% strain) with modulus of resilience of ~1 MPa, and continues with long (~100% strain) plastic deformation until breakage (Table 1). The structural rearrangements described above were probably the major contributors to the long plastic deformation of the single drawn fibers, where crosslink bonds between building blocks were probably breaking. In addition, these structural rearrangements may be the reason that the length and mechanics of the dry fibers have not been recovered.

The stiffness of single drawn fibers (~1 GPa) was lower than in the double drawn fibers (~1.5 GPa), which can be attributed to the direct stretching of the relatively aligned and strained protofilaments/paracrystalline fibers in the single drawn fibers, when compared to direct stretching of the non-oriented paracrystalline within pre-drawn fibers. In addition to the first modulus, two other distinct moduli were observed in all double drawn fiber curves, which is similar to the behavior of the human patellar tendon (Svensson et al., 2013). Together, these three distinct regions might correspond to the structural arrangements of the fiber during stretching. Namely, the modulus (~1.5 GPa) of the first region (0-4% strain) may describe the direct pulling of protofilaments/paracrystalline fibers. The second region (4-11% strain) modulus (~0.6 GPa) may describe the compaction and sliding of head to tail polymers along the protofilaments. The third region (11-21% strain) modulus (~0.3 GPa) may describe the uncoiling of the coiled coil dimers. This mechanical behavior can explain the reason for the limited extensibility (~20%) and higher breaking strength of the double drawn fibers (~120 MPa) when compared to single drawn fibers (100% and ~80 MPa). Overall, single drawn fibers (~55 MJ/m³) are tougher than double drawn fibers (~17 MJ/m³). The structural rearrangements in the second pull (single drawn

fibers) are mainly the compaction and slippage of the paracrystalline fibers and protofilaments. These rearrangements are probably the major reason for the higher breaking strain, and thus to higher toughness. Finally, the mechanical response of the wet fiber to tensile force leads us to predict that lamin wet fibers behave in a manner similar to soft and elastic fibers. It should be emphasized that the first pull started with wet fiber and ended when the fiber became dry, resulting in a force elevation. Generally, the wet fibers reached ~100% strain, which could attribute to the alignment and rearrangement of the microfibers network.

The mechanical properties of Ce-lamin macroscopic fibers are similar to other protein-based fibers that show hierarchical self-assembly of coiled coil protein (Table 2), such as, other IF-proteins and collagen. The properties of such materials can reach the excellent mechanical properties of spider silk fibers, as in the case of hagfish slime threads. The high strength and toughness of spider silk fiber arises from two repetitive alanine- and glycine-rich areas leading to crystalline (β -sheet) and amorphous (3_{10} -helices and β -turn spirals) structures (Heidebrecht and Scheibel, 2013), which contribute to the high stiffness and flexibility, respectively. Therefore, understanding the mechanism of secondary structure transition taking place in IF-protein-based fibers, which might contribute to the high strength, is highly important. To be precise, we need to understand how the difference in amino acids sequence between the proteins that built the hagfish slime threads and other IF protein-based fibers, such as lamin fibers, affects the hierarchical structure and thus the secondary structural transition mechanism.

4. Conclusions

In this study we have demonstrated that Ce-lamin assembles into tough macroscopic fibers with tensile properties similar to vimentin-based fibers, especially in their response to increased tensile force. Overall, lamins have shown a tendency to self-assemble into a dense fibrous structure that is utilized to produce materials with unique mechanical properties. These materials may render lamins into a good biomaterial in a variety of applications. Additionally, lamins play a major role in providing mechanoelastic protection to our genome by forming a composite filamentous network, the structure and mechanics of which are still unknown. In vitro, Ce-lamins assemble into native-like 10-nm filaments that are assembled from protofilaments. These filamentous structures are thought to exist within the nucleus (Mahamid et al., 2016). Paracrystalline fibers, which are the major building blocks of the macroscopic fiber, are organized similarly to Ce-lamin 10-nm filaments. Thus, the experimental setup described here can be utilized to study, in vitro, the mechanics of all lamin assemblies from different sources. In addition, this setup may produce another tool to investigate the structural-mechanical properties relationship in IF protein-based fibers by introduction of human disease-linked mutations (Worman and Bonne, 2007), which are known to deform the structure and mechanics of the cell nucleus and alter the structure of paracrystalline fibers in vitro (Taimen et al., 2009; Wiesel et al., 2008).

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Fig. 1. Ce-lamin macroscopic fiber and cryo-section of a microfibers network. (a) An electron micrograph projection from a tomographic reconstructed volume of a cryo-sectioned sample of a Ce-lamin microfibers network. Ce-lamin microfibers are comprised from paracrystalline fibers. Inset: Drawing of the Ce-lamin microfibers network inside the dialysis bag. (b) Ce-lamin wet macroscopic

fiber produced from a mechanically spun microfibers network. Inset: Drawing of the step showing the spinning of Ce-lamin microfibers into wet fiber.

Fig. 2. Structural organization of Ce-lamin fibers. (a) SEM images of Ce-lamin macroscopic fibers: pre-drawn, single drawn, and double drawn. Upper panel: higher magnification of lower panel. (b) Optical polarized microscopy of Ce-lamin macroscopic fibers: non-drawn, pre-drawn, single drawn and double drawn.

Fig. 3. Infrared absorption spectra of non-drawn (dash line) and double drawn (solid line) fibers in the amide I region. Each spectrum is an average of at least 15 measurements performed from different fibers and different sites along the fibers.

Fig. 4. Stress-strain curves of pre-drawn (green), single (blue), and double drawn (red) Ce-lamin fibers. All curves represent one fiber sample of the 8 fibers tested. Pre-drawn fiber test was stopped when the fibers became dry and force elevation was observed. Single and double drawn fibers showed a typical IF-response to tensile force. In general, double drawn fibers are stiffer but much less extensible.

Fig. 5. An assembly model of Ce-lamin dimers into macroscopic fibers. (a) Ce-lamin dimer and head-to-tail polymer of dimers. (b) Lateral association of two head-to-tail polymers into protofilament. (c) Lateral association of protofilaments into paracrystalline fibers. (d) A network of microfibers assembled from interconnected paracrystalline fibers spun into fiber. After stretching procedures, the paracrystalline fibers are oriented within Ce-lamin fibers.

Table 1. Average mechanical properties of Ce-lamin macroscopic fibers. The properties of pre-drawn, single, and double drawn fibers are displayed.

	Diameter (μm)	young's modulus (GPa)	break strain (d_L/L_0)	break stress (MPa)	Toughness (MJ/m^3)
Pre-drawn fibers	240 ± 19.3	0.003 ± 0.0001	1.05 ± 0.04	2.8 ± 0.26	1.71 ± 0.13
Single drawn fibers	217 ± 41.1	0.89 ± 0.32	1.01 ± 0.04	80.28 ± 11.73	54.83 ± 5.21
Double drawn fibers	185 ± 39.9	1.57 ± 0.17	0.21 ± 0.04	120.29 ± 26.46	16.73 ± 4.87

Table 2. Average mechanical properties of various biological fibers including the fibers tested in this study.

Protein	Break stress (MPa)	Young's modulus (GPa)	Break strain (d_L/L_0)
Keratin (wool) [1]	150	3	0.45
Hagfish slime (wet) [1]	180	0.006	2.2
Hagfish slime (dry) [1]	530	7.7	1
Hagfish (single drawn, dry)[2]	706	7.99	0.36
Vimentin + Mg^{2+} (single drawn) [3]	42	1.6	1.1
Vimentin + Mg^{2+} (double drawn) [3]	96	2.2	0.4
Dragline silk [4]	1469-544	22.1-4.5	0.36-0.17
Silkworm Silk [5]	248-165	6.10-3.82	0.33-0.19
Recombinant spider silk (96-mer) [6]	508	21	0.15
Human pataellar tendon (HPT) [7]	65	0.66	0.14
Ce lamin single drawn	80.3	0.89	1.01

Ce lamin double drawn	120	1.57	0.21
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[1] Ref. (Fudge and Gosline, 2004) [2] Ref. (Fudge et al., 2010) [3] Ref. (Fudge et al., 2010) [4] Ref. (Swanson et al., 2006) [5] Ref. (Cheung et al., 2009) [6] Ref. (Xia et al., 2010) [7] Ref. (Johnson et al., 1994).

Supplementary material

Fig. S1. Lamin microfibers network organized into a hollow cylinder-like structure within the dialysis bag at the second step of the dialysis procedure.

Graphical Abstract: